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IMMUNOFLUORESCENCE STUDIES IN THE DIAGNOSIS OF DERMATITIS HERPETIFORMIS AND ITS DIFFERENTIATION FROM BULLOUS PEMPHIGOID*

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ABSTRACT

Immunopathologic studies performed in the uninvolved skin in 19 typical cases of dermatitis herpetiformis (dh) revealed deposits of immunoglobulins at the dermal-epidermal junction. Either the only or the most predominant component was IgA. The pattern was microgranular, fibrillar or mixed. In 5 cases it was continuous along the basement membrane. While the only or most predominant component is IgG in bullous pemphigoid (bp), in dh, with a continuous IF pattern, IgA is invariably present and most prominent. Evaluation of IF studies, together with the clinical picture, histology, and response to sulfapyridine, points to the importance of immunopathology in the differentiation between dh and bp.

Immunofluorescence studies have revealed distinct differences between dermatitis herpetiformis Duhring (DH) and bullous pemphigoid (bp). This new line of evidence appeared to have confirmed the nosological distinctiveness of the latter. Circulating antibodies against the basement membrane of the epidermis are seen in a large proportion of the cases of bp. Fixed immunoglobulins in the same location are present in all cases (1, 2, 3, 4, 5, 6), and so is complement (7). Furthermore, Jordon *et al* (8) have found that some bp antibodies also fix complement *in vitro*.

In contrast to bp, neither circulating antibodies, immunoglobulins, nor complement have been found in the *area of lesions* in dh (3, 4, 6). However, the studies by van der Meer (9), which were made chiefly in the *unchanged skin*, have thrown new light on the immunological phenomena in dh. He demonstrated, in the epidermal-dermal junction, chiefly IgA, less commonly IgG, and complement. Fluorescence was granular in character, and a continuous line resembling that in bp was obtained only in one case.

Our purpose in the present work was to investigate the correlation between the immunopathological findings in dh and the macro and microscopic picture as well as responses to sulfapyridine. Most importantly, we sought to determine whether there are any differences in the composition of the immunoglobulins with which the patterns of the fluorescence, (continuous or granular) may be associated, and whether immunofluorescence studies offer a basis for differentiation between dh and bp.

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MATERALS AND METHODS

Our studies covered 19 cases of typical dh and 8 of suspected dh in which the clinical and histological findings were not diagnostically decisive. For comparison we chose 14 cases of bp, which were investigated in the same period and in which direct IF studies of fixed immunoglobulins *in vivo* also were performed. (Cases in which the sera only were examined were not considered).

The diagnostic criteria for dh were based on:

1) The clinical picture (erythemas, papules, and small vesicles characteristically distributed and in typical location)

2) histological features (microabscesses in the dermal papillae surrounding the bulla)

3) response to sulfapyridine, by which is meant a complete control of the disease, dramatic improvement with treatment, and relapses after the withdrawal of the drug.

The age of the patients, duration of the disease, and detailed data on its course are recorded in Table I. The data relating to the cases of bp are compiled in Table II.

Specimens of the unchanged skin around the lesions were used. They were frozen in solid carbon dioxide and sectioned to a thickness of 4 micra in a cryostat at a temperature of −20° C. The IF studies were made by the direct method with the aid of the following conjugates:

Conjugate A. Goat gamma-globulin anti-human IgG—16 units/1%P/ml; 3.4 mg Ab/ml; 10.5 mg

TABLE I

No	Age	Duration of the disease	Large bullae occasionally	Response to sulfapyridine	Histology microabscesses in dermal papillae	Skin lesions at time of IF investigations	Immunopathology					
							skin lesions			uninvolved skin		
							IgG	IgA	IgM	IgG	IgA	IgM
1. J.L.	26	6 yr	(−)	+	+	active	(−)	(−)	(−)	(−)	+++ granular	+++
2. C.S.	78	14 yr	(−)	+	(−)	abortive	(−)	(−)	(−)	(−)	+	
3. K.M.	60	8 yr	+	+	+	active	(−)	++ continuous	(−)	(−)	++ continuous	(−)
4. S.P.	34	4 yr	(−)	+	+	active				(−)	++++ granular	(−)
5. J.K.	54	6 yr	+	+	+	active	+	++++ continuous	+	−/+	+++/++ continuous	+++
6. H.S.	56	1 yr	+	+		active				+/+++	+++ granular	+
7. J.S.	66	10 mo	(−)	+		active				+	+++ continuous	++
8 B.K.	25	8 yr	(−)	+	+	abortive				+ in spots	+++ granular	(−)
9. W.R.	23	3 yr	(−)	+	+	abortive				±	++/+++ granular	±/(−)
10. S.S.	61	2 yr	(−)	+	+	active				(−)	++/+++ continuous	(−)
11. J.B.	50	6 mo	+	+	+	active	(−)	(−)	(−)	(−)	++ granular	+ in spots
12. M.M.	51	2 mo	(−)	+	+	active	+/++	+/++ continuous	(−)	++	+++ continuous	(−)
13. H.S.	21	3.5 yr	(−)	+		none				+ in spots	++ granular	
14. R.W.	37	3 yr	(−)	+	+	none				(−)	++ granular	(−)
15. K.O.	50	7 yr	(−)	+	+	none				(−)	+ granular	(−)
16. N.M.	46	1 yr	(−)	+	+	active	(−)	+++ granular		(−)	++ granular	
17. J.P.	51	23 yr	(−)	+		abortive	(−)	+++ granular		(−)	++ granular	
18. J.M.	21	1.5 yr	+	+		abortive				(−)	++ granular	
19. G.T.	31	2 yr	(−)	+		active				++	+++ granular	

TABLE II

No.	Age	Duration of the disease	Skin lesions	Circulating antibodies IF	Immunopathology			
					Skin lesions		Uninvolved skin	
					IgG	IgA	IgG	IgA
1. K.B.	70	2 yr	active	1/1280 - 1/80	+			
2. M.R.	77	3 yr	active	0	(-)		++++	
3. J.N.	80	5 mo	active	1/20	+++*			
4. R.W.	57	8 yr	active	0	++/+++			
5. P.P.	82	7 mo	active	0	+++		(-)	
6. S.K.	78	7 mo	active	0	+++	+++		
7. J.Ch.	70	6 mo	abortive	0	+++*			
8. M.K.	68	1.5 yr	active	0			+++	+
9. J.B.	80	3 mo	active	1/320 - 0	++/+++	(-)	+/++	(-)
10. S.M.	65	4 mo	active	1/80 organ and species specific			+++	(-)
11. W.P.	73	3 yr	active	1/40 - 0			+	(-)
12. Z.Z.	46	3 yr	active	1/80 - 0			+	(-)
13. J.K.	70	2 yr	none	0			(-)	(-)
14. E.S.	67	2 yr	none	1/80 - 0			(-)	(-)

* polyvalent conjugate/mixture of conjugates A, B, C.

P/ml; F/P molar—3.9. Dilution for use: ¼ U/ml (i.e. units/ml).

Conjugate B. Goat gamma-globulin-anti-human IgA—2 units/1%P/ml; 10.2 mg P/ml; F/P molar—2.9. Dilution for use ¼ U/ml.

Conjugate C. Goat gamma-globulin anti-human IgM—8 units/1%P/ml; 8.1 mg P/ml; F/P molar—2.6. Dilution for use: 1 U/ml. Conjugate not absorbed for light chains.

Conjugate D. Goat gamma-globulin anti-human fraction II Cohn—16 units/1% P/ml, 11 mg P/ml. F/P molar—5.5. Dilution for use: ½ U/ml. Rabbit or guinea pig mucosa served as the substrate. In the direct method, we used conjugate A and unrelated conjugate, goat anti-rabbit-gamma-globulins for control. Conjugates were prepared as described previously (16).

Specificity of the anti IgA conjugate was achieved by absorption. Tests on the absorbed conjugate were carried out with gel diffusion precipitation, using a chessboard type format. That is, serial dilutions of conjugate were tested against serial dilutions of isolated immunoglobulins IgG, IgA and IgM. Specificity of the anti IgG conjugates was achieved by dilution. That is, dilutions used for IF staining were found to be incapable of giving cross reactions with other immunoglobulins.

The IF results were read in a Fluorolume (American Optical) fluorescence microscope with exciting filter BG-12 and spare filter K-530.

RESULTS

In all the 19 cases of typical dh, immunoglobulins were demonstrated in the dermal-epidermal junction of the unchanged skin. Sometimes areas adjacent to the lesions failed to reveal typical IgA deposits, whereas more distant areas gave positive results. In very early erythematous lesions, IF was of the same nature as in the unchanged skin. In the areas of vesicular lesions, however, IF was usually absent or very faint and continued to the periphery.

In each case IgA was the principal or only component. IgG and possibly IgM also were present in five cases each, including three in which all three classes were present, but with IgA invariably predominating. Since the anti-IgM-conjugate was not absorbed for light chains, the presence of IgM needs to be verified by monospecific antiserum.

The IF was usually microgranular (Fig. 1) or fibrillar (Fig. 2) in character. Most intensive staining tended to occur in the tips of papillae. Occasionally it appeared throughout the papillae (Figure 3), usually with discontinuities in the interpapillary spaces. In eight cases investigated repeatedly, the IF reaction did not change between the different stages of the disease, not even after the active changes had subsided. In five cases IF was continuous along the basement membrane (Figure 4). In two of these five cases there were, at times, large pemphigoid-type bul-

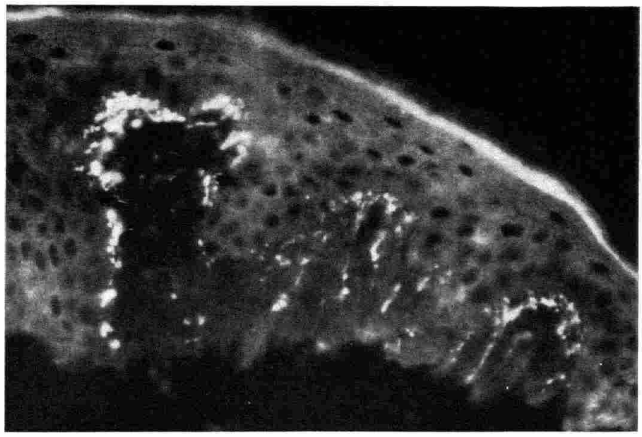

FIG. 1. Direct IF staining with monospecific anti-IgA conjugate. Specific granular fluorescence at the dermal-epidermal junction.

FIG. 2. The same stain as in Fig. 1 showing specific fibrillar fluorescence at the dermal-epidermal junction.

lae. However, large bullae also were observed during a few relapses in two cases in which the IF was of granular character.

Direct IF reactions with the conjugate of human bp serum also were negative at the floor of dh blisters, whereas they were eminently positive and showed a continuous pattern in the surrounding skin. In the unchanged skin, the double staining with the pemphigoid conjugate first and the anti-IgA conjugate second showed a continuous line along the basement membrane and granular deposits somewhat below.

In the 8 investigated atypical cases suspected of dh, the results were negative, and further observation also failed to confirm the tentative diagnosis.

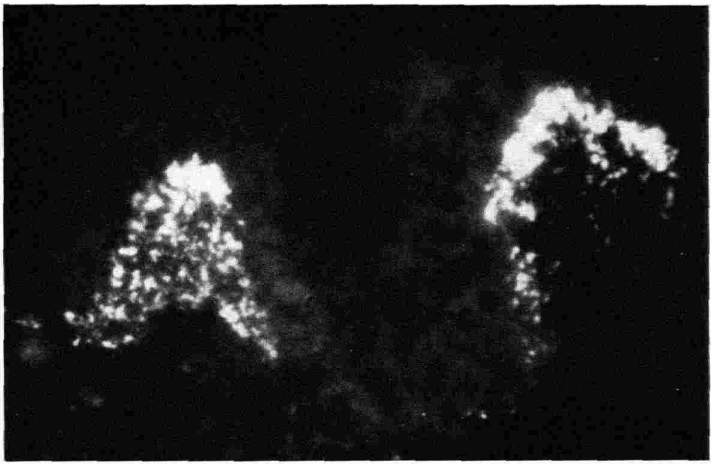

FIG. 3. Same staining as Figs. 1 and 2 showing microgranular fluorescence throughout the papillae.

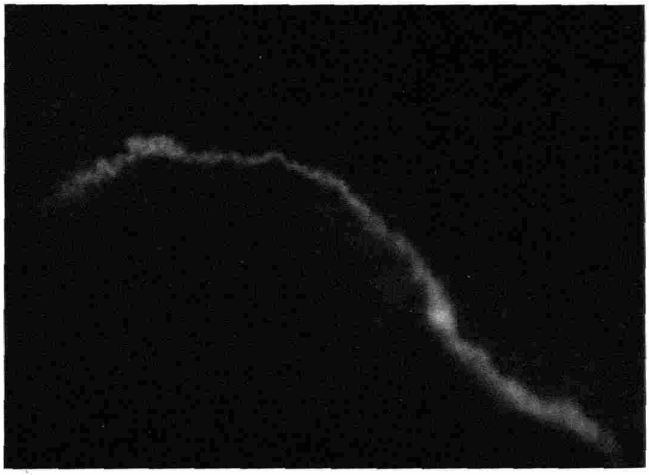

FIG. 4. Same staining as above showing continuous pattern of specific fluorescence along the basement membrane.

In the 14 cases of bp, in which IF studies were made on specimens either from the lesions or the unchanged skin, a continuous line along the basement membrane was invariably obtained. IgG invariably either predominated or was the only one present; usually the latter occurred. IF was positive both in the bullous lesions and normal skin. In two cases of bp, in which serial studies were made in the unchanged skin, IF-positive areas were found to have a focal distribution, i.e., some totally negative areas did occur.

It should be stressed that in serial sections of dh specimens, immunofluorescence was present

TABLE III

	Clinical features		Immunopathology							Response to sulfapyridine
						Patterns		Location in skin		
	Small vesicles and erythematous patches (typical)	Large bullae	IgA	IgM	IgG	Dermal-epidermal-junction	Papilla	Normal (distal to bulla)	At or near fresh bulla or vesicles	
D.H.	always*	rare	all*, [1]	some?[1]	some[1]	microgranular, fibrillar; continuous	microgranular; fibrillar	Positive	Usually negative	always dramatic*
B.P.	in some cases erythematous plaques	always*	some[2]	some[2]	all*, [2]	continuous	none	Variable	Always positive	some in few cases[5]?
SLE	none[3]	sometimes (of erythema multiforme type)[3]	some[4]	some[4]	mostly[4]	similar to d.h. but tends to give coarse granules; in some cases similar to b.p.	similar to d.h. but tends to give coarse granules	Variable	Positive	none

* All or always means: in all cases observed up to the present time.

[1] distant from lesions, and not at the lesions.

[2] at the lesions or in their vicinity: frequently also at distant sites.

[3] clinical and histological picture is entirely different and does not pose any problem in the clinic.

[4] according to Cormane *et al.* (1966) (15).

[5] See text.

in some areas only; some sections had negative reactions.

DISCUSSION

The present investigations made with a cardioid darkfield condenser fully confirm the reports by van der Meer (9) and Cormane *et al.* (10). Namely, in dh there are immunoglobulins, especially IgA, in the dermal papillae of the seemingly unchanged skin. In cases in which IgG was detected in addition to IgA, the latter invariably predominated. The presence of IgM calls for further investigations with monospecific conjugate, and will not be discussed here.

The character of the fluorescence in dh can be classified in the following order of frequency: (See Table III).

1) microgranular, often seen throughout the dermal papilla
2) fibrillar, distributed close to and along the basement membrane
3) homogeneous, visible as a continuous line along the basement membrane

Occasionally a mixed microgranular-fibrillar pattern was observed. In one case a granular pattern appeared in a few foci with the continuous linear pattern predominating in the whole specimen. The character of IF did not change between remissions and relapses in particular cases. Irrespective of the IF pattern, IgA predominated invariably, thus distinguishing the continuous fluorescence in dh from that in bp.

Another difference in comparison with bp is the absence of IF in the vesicular lesions in dh. In bp, on the other hand, linear staining for IgG is usually seen even along the dermal papillae which form the floor of bulla.

Using the direct technique with labeled bp antibodies we could show fibrillar and microgranular IF to have no immediate connection with the basement membrane. In contrast, continuous IF was indistinguishable from that in bp.

The method also made it possible to demonstrate that the basement membrane containing antigen reacting with bp antibodies is completely destroyed in the vesicular lesions in dh. The granular IF observed in LE (11, 12, 13, 14) in general tends to have a somewhat different character; it is usually more coarse-grained. However, if IF shows microgranular or fibrillar deposits of IgA predominantly, its pattern is usually diagnostic for dh. Indeed, the microgranular deposits throughout the papillae which occur in some cases of dh seem to be characteristic of this condition. The fibrillar IF, which occurs less frequently in dh, may resemble thread-like IF pattern in LE (11).

This is in contrast to bp where fresh lesions always contain the antigen and only the old lesions show the loss thereof.

The studies have shown that there are IF features characteristic of dh in cases which meet the strict diagnostic criteria based on the macro and microscopic picture and response to sulfapyridine.

Unfortunately, misleading observations on the action of sulfapyridine derive from the fact that the patient is not observed over a sufficient period of time. Sometimes patients have spontaneous remissions at the time of sulfapyridine treatment. In these cases withdrawal of the drug is usually not accompanied by relapse. However, in a true response to sulfapyridine, relapses invariably follow withdrawal of the drug. Adequate doses of the drug will control the disease.

The continuous line differs between dh and bp only in immunoglobulin composition. Cases with this type of IF were no different from those with granular or fibrillar IF either in course, clinical features, or response to sulfapyridine. In two of them there appeared, in occasional relapses, large bullae as in bp, but the same was observed in some relapses in two other cases in which IF was granular. However, unlike bp, we have never found circulating antibodies against the basement membrane in cases with a continuous IF line and IgA as the principal immunoglobulin.

Judging by these results, immunofluorescent studies seem to offer a basis for differentiation between dh and bp as summarized in Table III. This differentiation is especially important in cases of dh in which major bullae suggestive of bullous pemphigoid appear in some relapses.

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